Haemophilus ducreyi as a cause of skin ulcers in children from a yaws-endemic area of Papua New Guinea: a prospective cohort study

Oriol Mitjà, Sheila A Lukehart, Gideon Pokowas, Penias Moses, August Kapa, Charmie Godornes, Jennifer Robson, Sarah Cherian, Wendy Houinei, Walter Kazadi, Peter Siba, Elisa de Lazzari, Quique Bassat

Summary

Background Skin infections with ulceration are a major health problem in countries of the south Pacific region. Yaws, caused by *Treponema pallidum* subspecies *pertenue* and diagnosed by the presence of skin ulcers and a reactive syphilis serology, is one major cause, but this infection can be confused clinically with ulcers due to other causative agents. We investigated *T pallidum pertenue* and another bacterium known to cause skin infections in the Pacific islands—*Haemophilus ducreyi*—as causes of skin ulceration in a yaws-endemic region. Additionally, we identified specific signs and symptoms associated with these causative agents of cutaneous ulcers and compared these findings with laboratory-based diagnoses.

Methods We did a prospective cohort study of five yaws-endemic villages (total population 3117 people) during a yaws elimination campaign in Papua New Guinea in April, 2013. We enrolled all consenting patients with chronic moist or exudative skin ulcers. We undertook a detailed dermatological assessment, syphilis serology, and PCR on lesional swabs to detect the presence of *T pallidum pertenue* and *H ducreyi*. Patients with PCR-confirmed bacterial infections were included in a comparative analysis of demographics and clinical features.

Findings Full outcome data were available for 90 people with skin ulcers. Of these patients, 17 (19%) had negative results in all molecular tests and were therefore excluded from the comparative analyses. A bacterial cause was identified in 73 (81%) participants—either *H ducreyi* (n=42), *T pallidum pertenue* (yaws; n=19), or coinfection with both organisms (dual infection; n=12). The demographic characteristics of the patients infected with yaws and with *H ducreyi* were similar. Skin lesions in patients with yaws and in those with dual infection were larger than those in patients infected with *H ducreyi* (p=0·071). The lesions in patients with yaws and dual infection were more circular in shape (79% and 67%) than in those infected with *H ducreyi* (21%; p<0·0001); more likely to have central granulating tissue (90% and 67% *vs* 14%; p<0·0001); and more likely to have indurated edges (74% and 83% *vs* 31%; p=0·0003). The prevalence of reactive combined serology (positive *T pallidum* haemagglutination test and rapid plasmin reagin titre of ≥1:8) was higher in cases of yaws (63%) and dual infections (92%) than in *H ducreyi* infections (29%; p<0·0001).

Interpretation In this yaws-endemic community, *H ducreyi* is an important and previously unrecognised cause of chronic skin ulceration. Reactive syphilis serology caused by latent yaws can occur in ulcers with the presence of *H ducreyi* alone. The introduction of PCR for ulcer surveillance could improve the accuracy of diagnosis in countries with yaws eradication campaigns.

Funding Newcrest Mining Company.

Lancet Glob Health 2014; 2: e235–241

See **Comment** page e187

Barcelona Centre for International Health Research, Hospital Clinic, University of Barcelona, Barcelona, Spain (O Mitjà MD, E de Lazzari MSc, Q Bassat MD); **Lihir Medical Centre – International SOS, Newcrest Mining, Lihir Island, Papua New Guinea** (O Mitjà, G Pokowas HEO, P Moses HEO, A Kapa BSc) **Department of Medicine, University of Washington, Seattle, WA, USA** (Prof S A Lukehart PhD, C Godornes BSc); **Department of Global Health, University of Washington, Seattle, WA, USA** (Prof S A Lukehart); **Department of Microbiology, Sullivan Nicolaides Pathology, Brisbane, Australia** (J Robson MD, S Cherian MD); **Disease Control Branch, National Department of Health, Port Moresby, Papua New Guinea** (W Houinei HEO); **World Health Organization, Office of the WHO Representative, Boroko NCD, Port Moresby, Papua New Guinea** (W Kazadi MD); **and Papua New Guinea Institute of Medical Research, Goroka, Eastern Highland Province, Papua New Guinea** (Prof P Siba PhD)

Correspondence to:
Dr Oriol Mitjà, Department of Community Health, Lihir Medical Centre, Post Office Box 34, Lihir Island, NIP, Papua New Guinea
oriolmitja@hotmail.com

Introduction

Chronic skin infections with ulceration are an important cause of morbidity in children in the south Pacific. In many countries in the region, one of the most common causes is yaws—a chronic relapsing non-venereal treponematosis that is highly contagious and is transmitted by skin-to-skin contact.[1] It is caused by the bacterium *Treponema pallidum* subspecies *pertenue*. Patients are diagnosed with this infection if non-treponemal and treponemal serological tests are reactive. However, asymptomatic seroreactivity caused by latent yaws infection is common, and occurs in up to 30% of children in the region.[2,3] Therefore, other possible causes of skin ulcer should be investigated, even in patients who are seroreactive for treponemal infection. Little attention has been paid to the possible role of *Haemophilus ducreyi* in chronic skin ulcers.[4–7] Previously, *H ducreyi* was a very common cause of a genital ulcer, called chancroid, in many developing countries, although rates have fallen sharply in sub-Saharan Africa and elsewhere as a consequence of syndromic management of genital ulcer disease. Non-sexually transmitted *H ducreyi*, which manifests as lower leg lesions in the absence of genital lesions, was first described in 1989 in a 22-year-old man who had visited Fiji,[4] and six additional cases have been reported in

people who had been to Samoa, Papua New Guinea, or Vanuatu (figure 1).[5–7] No cases have been reported outside the south Pacific. *H ducreyi* has not been recognised as a common cause of skin ulceration, probably because of the absence of serological testing for the bacterium and its fastidious culture requirements, which call for technical expertise beyond the capabilities of most routine diagnostic laboratories in developing countries.

In patients with yaws-like skin ulcers, clinical differentiation of other bacterial causes other than *T pallidum pertenue* has important implications for diagnostic approach, case reporting, case management, and prevention strategies in Papua New Guinea and elsewhere. For example, accurate identification of cases is crucial for the ongoing WHO-directed yaws eradication campaign according to the Morges strategy: initial mass treatment, followed by surveys every 6 months to detect and treat remaining cases of yaws.[8] For this strategy to be effective, active cases of yaws must be accurately detected.

We used molecular assays to investigate *H ducreyi* and *T pallidum pertenue* as causes of skin ulceration in a yaws-endemic region of Papua New Guinea. Additionally, we identified specific signs and symptoms associated with these causative agents of cutaneous ulcers, and compared these findings with laboratory-based diagnoses.

Methods

Participants

We did a prospective cohort study of the population in a cluster of five villages (Zuen, Kunaye-2, Matakues, Komat-2, and Wurtol; total population 3117 people) in Lihir Island, Papua New Guinea, during a yaws elimination campaign in April, 2013. All villages in Lihir Island have a high prevalence of yaws[9] and the population had not received mass drug administration with azithromycin before we obtained swab samples from participants.

We enrolled consecutive, untreated, consenting people with chronic (symptomatic for >2 weeks) atraumatic skin ulcers. This case definition is consistent with the surveillance case definition for yaws provided by WHO.[10] In this study, only participants with an exudative ulcer were included; thus, patients whose ulcers were dry and crusted or had re-epithelialised were not eligible. Enrolled patients who did not have an established molecular diagnosis on PCR were excluded from subsequent comparative analyses. Participants with two PCR-defined infections were classified as dual infections in a separate group.

All participants, or their parents or guardians, provided oral informed consent to be screened and treated during the elimination campaign. Additionally, we obtained written informed consent from people with lesions before enrolment in this study; all eligible patients consented to enrolment. The protocol was approved by the National Medical Research Advisory Committee of the Papua New Guinea Ministry of Health (MRAC no. 12.36).

Procedures

We initially did a census of every household, after which the villagers underwent screening assessment of the face, arms, and legs, for which we used the simplified system of the WHO yaws pictorial guide.[10] The pictorial guide

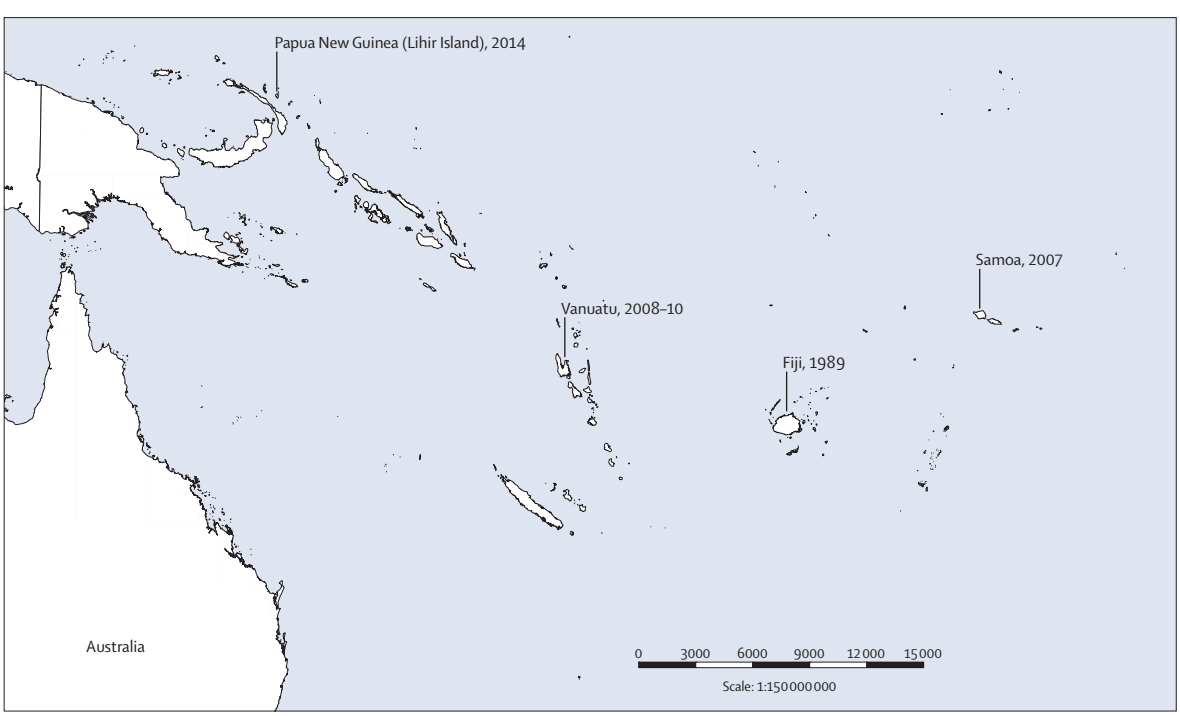

Figure 1: **Distribution of cases of *Haemophilus ducreyi* skin ulcers reported in the south Pacific countries since 1989**

includes five signs: papilloma, ulcers, squamous macules, bone lesions, and plantar hyperkeratosis. Participants with yaws-like ulcers were assessed further. We recorded information about every participant on a specifically designed data collection form. A medical history was obtained and a detailed dermatological examination of the whole body, except for the genital region, was done by an experienced clinician (OM, GP, or PM). A photograph of the skin lesion(s) was taken. The clinician measured and recorded the size and shape of the ulcers, and their depth, location, and tenderness. Characteristics of the bases and borders of the ulcers were also noted, including the induration of the border. We did not record the presence or absence of regional lymphadenopathy close to the lesion. The investigators standardised objective criteria for the quantitative assessment of these characteristics. The largest ulcer present on each patient was measured along its major and minor axes, and a smaller than 20% difference between axes was used to confirm the impression obtained by visual inspection as to the circular shape of ulcers. We judged an ulcer to be deep if the epithelium was denuded and the ulcer also involved the subcutaneous tissue. Tenderness was reported if there was an involuntary pain reaction upon palpation. We recorded the duration of the lesions, and we stratified participants into two groups—less than 4 weeks' duration or 4 weeks or longer—on the basis of a previous finding that treponemal serological tests become positive within 4 weeks of the appearance of the lesion. Every participant with a suspected yaws ulcer received azithromycin treatment (30 mg/kg single oral dose) for yaws[11] and a simple dry dressing was applied to keep ulcerated lesions clean and protected from trauma.

Laboratory assessment

We collected blood samples to determine the rapid plasma reagin titre and to complete the *T pallidum* haemagglutination test. Syphilis serology was judged to be diagnostic for treponemal infection if the *T pallidum* haemagglutination test was positive and the rapid plasma reagin was reactive (titre ≥1:8). Rapid plasma reagin titres of 1:8 or higher have been viewed as the marker for true infection in previous yaws studies.[12]

The exudate from the largest ulcer on each patient was collected by vigorous rubbing of the base of the lesion with two sterile cotton-tipped swabs that were inserted into prelabelled cryotubes containing 0·5 mL transport medium to lyse the organisms and optimise DNA stability; the swabs were then frozen (at –20°C) for transport to the laboratories. The first lesion specimens were sent to the University of Washington laboratory (Seattle, WA, USA) for molecular testing with PCR to detect *T pallidum* DNA (to confirm treponemal infection), a molecular signature specific to the subspecies *pertenue* (to confirm yaws infection), and evidence of mutations conferring resistance to azithromycin. As described previously,[13] DNA was extracted under stringent PCR-clean conditions to avoid DNA contamination of the samples. We used three *T pallidum* gene targets to detect *T pallidum* DNA—*tp054*, *tpN47* (*tp0574*), and *tprL* (*tp1031*)[13–16]—and judged a sample to be *T pallidum* positive if at least two of the three gene targets could be amplified. All samples not amplifiable for *T pallidum* DNA were confirmed as negative by *tpN47* Southern blot. To distinguish between treponemes that cause yaws (*T pallidum pertenue*) or syphilis (*T pallidum pallidum*), a specific region of *tprL* was PCR amplified with Promega Hot Start Taq (Promega, Madison, WI, USA), and the subspecies distinction was established as described previously.[16] Detection of the macrolide resistance mutations (A2058G and A2059G) in the 23S rRNA gene was done by PCR and amplicon restriction digestion as previously described.[13]

The second swab was forwarded for molecular testing for *H ducreyi* to the Molecular Diagnostic Unit at Queensland Royal Brisbane and Women's Hospital (QLD, Australia). Nucleic acids were purified on the MagNA Pure96 (Roche Diagnostics, Indianapolis, IN, USA) with use of 200 µL of each resuspended specimen in the DNA and Viral NA Small Volume Kit (Roche Diagnostics). Taqman real-time PCR targeting the 16S rRNA gene used previously published primers and probes.[17]

Statistical analysis

We compared the clinical features and serological results of the participants with *H ducreyi*, yaws, and dual infections with Fisher's exact test. We tested differences in median values for continuous measurements between the groups with the Mann-Whitney *U* test. We did further subgroup analyses to assess the serological results by duration of disease in participants with positive *T pallidum pertenue* PCR. We reported odds ratios with 95% CIs from multinomial logistic regression to compare the clinical characteristics and physical examination findings for ulcers caused by the different bacteria. All analyses were done with Stata version 13.1.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full

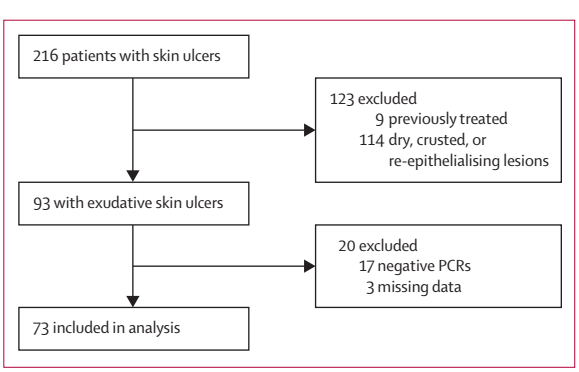

Figure 2: **Study profile**

	Yaws cases (n=19)	Dual infection cases (n=12)	*Haemophilus ducreyi* cases (n=42)	Yaws *vs H ducreyi*: unadjusted OR (95% CI)	Dual infection *vs H ducreyi*: unadjusted OR (95% CI)	p value (multinomial model)*
Age (years)	10 (8–14)	7 (4–13)	10 (7–14)	1·01 (0·94–1·07)	0·89 (0·78–1·02)	0·143
Number of children aged <15 years	15 (79%)	12 (100%)	33 (79%)	1·02 (0·27–3·85)	NA	0·074
Male sex	11 (58%)	7 (58%)	27 (64%)	0·76 (0·3–2·3)	0·78 (0·21–2·88)	0·864
Characteristics of lesions						
Duration (weeks)	4 (3–6)	4 (3–7)	3·5 (3-4)	1·01 (0·85–1·20)	1·05 (0·87–1·27)	0·861
Diameter >2 cm	14 (74%)	8 (67%)	18 (42·9%)	3·35 (1·06–10·62)	2·45 (0·66–9·11)	0·071
Largest diameter of lesion (cm)	2·2 (1·8–2·8)	2·1 (0·8–2·4)	1·6 (1·0–3·0)	NA	NA	0·087
Smallest diameter of lesion (cm)	2·2 (1·2–2·5)	1·9 (0·6–2·2)	1·4 (0·6–1·8)	NA	NA	0·002
Round shape	15 (79%)	8 (67%)	9 (21%)	13·75 (3·65–51·81)	7·33 (1·79–29·99)	<0·0001
Uniform colour with granulating bed	17 (90%)	8 (67%)	6 (14%)	51·00 (9·31–279·45)	12·00 (2·73–52·66)	<0·0001
Indurated edges	14 (74%)	10 (83%)	13 (31%)	6·25 (1·86–21·00)	11·15 (2·14–58·26)	0·0003
Deep ulcers	19 (100%)	11 (92%)	11 (26%)	NA	31·00 (3·58–268·69)	<0·0001
Tender lesions	8 (42%)	8 (67%)	32 (76%)	0·23 (0·1–0·7)	0·6 (0·2–2·5)	0·084
Lesion site in lower extremity	19 (100%)	12 (100%)	34 (81%)	NA	NA	0·175

Data are n (%) or median (IQR), unless otherwise indicated. 17 participants not included in this table were negative in all molecular tests. OR=odds ratio. NA=not applicable. *p value for multinominal logistic regression; *H ducreyi* is the base outcome.

Table 1: **Demographic characteristics, clinical features, and results of physical ulcer examination, by bacterial cause**

Figure 3: **Appearance of leg ulcers in children from Lihir Island**
(A, B) Shallow, slough-base ulcers with rolling edges in patients with *Haemophilus ducreyi* infection. (C, D) Red, moist, hypergranulating ulcers with raised edges on the legs of patients with primary yaws.

access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During the community survey, 3117 people lived in the area, of whom 2867 underwent initial assessment. We identified 216 people with skin ulcers, of whom 93 were judged to have exudative lesions (figure 2). Three patients were excluded because of incomplete data, so a total of 90 people were enrolled. 17 people (19%) were negative in all molecular tests (PCRs) and were excluded from subsequent comparative analyses.

Of 90 participants tested, 54 (60%) were positive for *H ducreyi* on real-time PCR. *T pallidum* PCR was positive at several gene targets in 31 (34%) specimens, and *tpN47* Southern blot was used to confirm *T pallidum*-negative samples. All 31 confirmed positive specimens had *tprL* molecular signatures that identified them as subspecies *pertenue*, and all *T pallidum* samples had wild-type 23S, which confirms susceptibility to azithromycin. All samples had amplifiable human β-globin DNA, used as a control for DNA integrity and adequacy of sample.

A bacterial cause was identified by PCR amplification techniques in 73 (81%) of 90 case participants. Of these 73 patients, *H ducreyi* alone was identified in 42 (58%), *T pallidum pertenue* alone in 19 (26%), and co-infection with both organisms in 12 (16%). The demographic characteristics of the participants with *H ducreyi*, yaws, and dual infection were similar (table 1). The age of people with skin ulcers ranged from 2 to 40 years, but ulcerative lesions occurred mainly in children and young adults (median age 10·0 years, IQR 6·5–14). The sex ratios in the study groups were similar, and overall 62% were male.

When we compared individual clinical signs, ulcers with a known single treponemal infection were similar to those of dual infection with yaws and *H ducreyi*, and differed significantly from *H ducreyi* ulcers; however, we noted some overlap in clinical characteristics between the first two groups and the latter one. On physical examination, the largest median diameters and smallest median diameters of the skin ulcers were significantly smaller for cases of *H ducreyi* than for cases of yaws (table 1). The yaws and dual-infected lesions were more

circular in shape than were *H ducreyi* ulcers (table 1), as assessed by visual inspection and as confirmed by measurement of the difference in dimensions between the largest and smallest diameters of the skin lesions. Skin lesions in patients with yaws and dual infection were more likely to show central granulating tissue and indurated edges (table 1, figure 3), and were more often deep, involving subcutaneous tissue (table 1), than were those in patients with *H ducreyi* infection (table 1). Large upper limits of the 95% CIs for the variables of granulating bed and ulcer depth (table 1) are indicative of the frequency distribution of the underlying contingency tables. Yaws lesions caused by *T pallidum pertenue* alone were less likely to be tender than were lesions containing *H ducreyi* as the sole or co-pathogen (table 1). None of the ulcers caused by either *T pallidum pertenue* or *H ducreyi* showed signs of cellulitis around the lesion.

Of the 73 participants with causative bacterial diagnoses, 35 (48%) presented with a combination of positive *T pallidum* haemagglutination test and rapid plasmin reagin titre of 1:8 or higher, and 26 (36%) had a rapid plasmin reagin titre of 1:32 or higher (table 2). Combined positive serology with a rapid plasmin reagin titre of 1:8 or higher occurred more frequently in cases of yaws and dual infection than in patients with *H ducreyi* infection alone (table 2). 12 of 42 (29%) participants whose PCR was positive for *H ducreyi* and negative for *T pallidum* had serological tests positive for treponemal infection, and ten (24%) had rapid plasma regain results at high titre (table 2). Because coincident false-positive treponemal and non-treponemal tests are quite rare, these patients probably had latent yaws infection, with the lesion caused by *H ducreyi*. PCR of blood for *T pallidum* might be able to identify persistent yaws treponemes in these patients.

Seven of 19 (37%) participants with positive *T pallidum pertenue* PCR and one of 12 (8%) patients with dual infection had non-reactive serological tests for treponemal infection. Seven of these eight patients had an ulcer for up to 3 weeks and might have been in the very early pre-seroconversion stage of yaws. In subgroup analyses by duration of disease, the reactivity of combined serological tests in patients with yaws or dual ulcers for 4 weeks or longer was significantly higher than that in patients with ulcers for 3 weeks or less (95·2% *vs* 30%; p=0·0003).

Discussion

In this yaws-endemic region of Papua New Guinea, we expected that the chronic ulcers in children younger than 15 years would be mainly caused by *T pallidum pertenue*. However, our molecular assessment of ulcer cause showed that *H ducreyi* is a very common trigger of chronic skin ulcer. Almost three-quarters of the patients who presented with skin ulcers had *H ducreyi* detectable in the lesions by PCR, whereas *T pallidum pertenue* DNA was detected in almost half of participants. 16% of participants had *H ducreyi* and *T pallidum pertenue* detectable, which suggests dual infection.

Human-to-human transmission of *H ducreyi* has long been thought to be mainly through sexual contact.[18] Chancroid, caused by *H ducreyi*, was a leading cause of genital ulcer disease in sub-Saharan Africa in the 1980–90s, and was also quite common in several Asian countries.[19–23] The introduction of syndromic treatment of patients with genital ulcers (treatment of genital ulcers with antibiotics effective against both syphilis and chancroid) has led to large reductions in syphilis and the virtual disappearance of chancroid in sub-Saharan Africa. At present, the prevalence of genital ulcers in Lihir Island is low. The sexually transmitted infections surveillance system relies on reporting of cases with laboratory confirmation, and in 2012 the number of new syphilis cases was 20 cases (1·4 per 1000 people). Very few data exist for the incidence of chancroid in the south Pacific, although syndromic management of genital ulcer disease has been practised in these countries since 2002.[24]

Non-sexual transmission of *H ducreyi* has recently been described in six people who developed skin ulcers while visiting south Pacific countries.[5–7] Non-genital skin lesions can occur in patients with chancroid as a result of autoinoculation,[25] and human experimental models involving inoculation of the dermis of the forearm have shown that *H ducreyi* can infect the skin.[26,27] In our study, about 2% of the total population and more than 7% of children aged 5–15 years seem to suffer from non-sexually transmitted *H ducreyi* skin infection. These unexpected, but molecularly confirmed, findings warrant consideration of this bacterium in people from this region who have chronic ulcers. In the face of effective treatment against genital infection, the hypothesis that *H ducreyi* has found

	Yaws cases (n=19)	Dual infection cases (n=12)	*Haemophilus ducreyi* cases (n=42)	Yaws *vs H ducreyi*: unadjusted OR (95% CI)	Dual infection *vs H ducreyi*: unadjusted OR (95% CI)	p value (multinomial model)*
TPHA	14 (74%)	11 (92%)	26 (62%)	1·72 (0·52–5·70)	6·77 (0·80–57·52)	0·093
RPR	14 (74%)	11 (92%)	22 (52%)	2·55 (0·78–8·34)	10·00 (1·18–84·55)	0·017
Combined serology (RPR titre ≥8)	12 (63%)	11 (92%)	12 (29%)	4·29 (1·36–13·50)	27·50 (3·19–236·98)	<0·0001
High-titre serology (RPR titre ≥32)	9 (47%)	7 (58%)	10 (24%)	2·88 (0·91–9·07)	4·48 (1·16–17·27)	0·041

Data are n (%), unless otherwise indicated. 17 participants not included in this table were negative in all molecular tests. OR=odds ratio. TPHA=*Treponema pallidum* haemagglutination test. RPR=rapid plasma reagin. *p value for multinominal logistic regression; *H ducreyi* is the base outcome.

Table 2: **Serological results, by bacterial cause**

a new niche in the skin of children is interesting. An analogy could be made to the case of *T pallidum*'s role in causing the genital ulcer disease syphilis in sexually active adults and the non-venereal skin ulcerations seen as yaws in children. Different subspecies of *T pallidum* are associated with yaws and syphilis, and genomic comparisons of *H ducreyi* from genital ulcers versus skin lesions are important. We intend to undertake additional investigations of such lesions, including cultures to help molecular characterisation of the *H ducreyi* strains and their microbial susceptibility.

Our study describes, for the first time, specific characteristics that might improve the clinical diagnosis of skin ulcers in children and young adults in the south Pacific (panel). The dermatological findings presented indicate that several prominent clinical differences exist between yaws and *H ducreyi* skin ulcers in the tropics. Particular individual signs, such as granulating-based ulcers with indurated edges in the case of yaws, and shallow slough ulcers in the case of *H ducreyi*, have adequate specificity and might prove helpful to clinicians, although some overlap in clinical manifestations does occur.

Our comparative analysis has some limitations. First, it was done in only one area of a single island in the Pacific. Therefore, whether the clinical findings can be extrapolated to cases of skin ulcers in other regions within the Pacific or to other regions in the tropics that report cases of yaws, is unclear. However, the observations about *H ducreyi* ulcers in this study are comparable to those made in other case reports from Fiji, Samoa, and Vanuatu.[4–7] Second, other pathogens, such as *Mycobacterium ulcerans*, other non-tuberculous mycobacteria, *Corynebacterium diphtheriae*, and *Arcanobacterium haemolyticum*, can cause chronic ulcers in the tropics and were not sought in this study. Third, the clinical measures are dependent on the skill of the clinician and are thus affected by factors including previous experience, knowledge of epidemiological risk factors, and disease prevalence. Therefore, the accuracy of clinical diagnosis could vary greatly in different clinics, and surveillance for yaws based on clinical case definitions alone (in the absence of objective laboratory data) seems to be unreliable.

Serological testing has been used to help in the diagnosis of yaws infections. Traditionally, patients in whom seropositivity was associated with the presence of skin ulcers were presumed to have yaws. However, in our study, 12 (34·2%) of 35 case participants with skin ulcer and rapid plasmin reagin titres of 1:8 or higher had monoinfection with *H ducreyi* according to the PCR results. Without our detailed investigation, the skin ulcers of all children and adults who met the case definition of yaws and had positive syphilis serology would have been attributed solely to treponemal infection. These serological results, with use of both lipoidal and treponemal tests, indicate that the underlying prevalence of *T pallidum* infection (active and latent) in these communities might be quite high. The proposed use of a mass treatment approach for yaws eradication, in which all community members are treated, is strongly supported by evidence of high seroprevalence.

Conversely, an absence of seropositivity should not be interpreted as exclusion of the presence of yaws infection. The primary chancre of syphilis can be present for days to weeks before seroconversion occurs, and the same is true of yaws. In our study, *T pallidum* serology was non-reactive in eight patients (25·8%) in whom yaws infection was proven by PCR, and many of these had lesions of shorter duration, presumably detected at such an early stage of the infection that seroconversion had not yet occurred. *T pallidum* PCR performance in early infection was more informative than syphilis serology. We noted that individuals with *T pallidum pertenue* and *H ducreyi* co-infection were more likely to be seropositive than were those with either infection alone, raising the possibility that these were chronic yaws lesions that had been superinfected by *H ducreyi*.

In our study, molecular techniques have been used for the first time to measure the prevalence of active yaws. Use of this technology had previously been reported in only one suspected yaws case.[28] We used molecular signatures to confirm that the *T pallidum* present in these lesions was subspecies *pertenue*, which is an important consideration in geographical regions where both venereal and non-venereal transmission might occur in close proximity. Although no *T pallidum* PCR has been approved by the US Food and Drug Administration, in-house assays are available in many large laboratories. Use of such a test could shorten turnaround time and improve diagnostic accuracy in yaws surveillance programmes.

In the planning and implementation of a multinational yaws eradication campaign using azithromycin, a comprehensive strategy for the diagnosis and control of skin infections needs to be developed. First, we should explore the possibility of the use of DNA technology to investigate the causes of lesions in regions around the world, since this approach would improve the detection

***Panel:* Research in context**

Systematic review

We systematically searched PubMed up to April 1, 2013, with the search terms "skin ulceration", "infection", "bacteria", and "Pacific". Yaws and Buruli ulcer have been widely studied as a cause of skin ulcer in the south Pacific. We identified four case reports that showed that *Haemophilus ducreyi* is a cause of chronic skin ulceration in patients returning from Pacific island countries.[4–7] However we did not identify any study that analysed the prevalence of this causative agent in skin ulcers.

Interpretation

We studied children and young adults with chronic skin ulcers for *T pallidum pertenue* and *H ducreyi* with molecular assays. This study provides substantial evidence that *H ducreyi* is a common cause of skin ulcer in a yaws-endemic community in the south Pacific region and might have a distinct clinical presentation from that of yaws. This finding indicates that consideration of this bacterium is warranted in people from the region who have chronic ulcers and has important implications for the diagnostic approach and prevention strategies during implementation of the multinational yaws eradication campaign.

accuracy and treatment of remaining yaws cases during the elimination phase. In addition to *H ducreyi* described here, cutaneous leishmaniasis, Buruli ulcer, and cutaneous diphtheria are all included in the differential diagnosis of yaws. Training and technology transfer for PCR assays for the detection of *T pallidum pertenue* and *H ducreyi* should be supported. Of note, 1 year after mass treatment, the number of asymptomatic seroreactors will be very low. Therefore, the correlation between positive syphilis serology and *T pallidum pertenue* molecular results should be stronger than that reported here, which should allow the serological test to better differentiate yaws and *H ducreyi*. Second, mass administration of azithromycin to eradicate yaws might have beneficial secondary effects, including a reduction in the number of cases with *H ducreyi*. The recommended treatment for *H ducreyi* infection includes azithromycin 1 g orally in a single dose.[29] Further assessment of the consequences of mass oral distribution of azithromycin on *H ducreyi* prevalence and of bacterial resistance to macrolides will be needed to help guide public health decision making.

In summary, *H ducreyi* is an emerging or previously unrecognised causative agent in chronic skin ulcers in children and young adults from Papua New Guinea. Recognition of the several causes of skin ulcers and raised clinical acumen are needed to enable specific diagnosis. However, important overlap exists in the clinical manifestations of yaws and *H ducreyi* skin ulcers, and reactive serological results for treponemal infection can occur in the presence of lesions that contain *H ducreyi* alone. In this very complicated setting, PCR techniques should be used as a quality control measure in yaws eradication programmes.

Contributors

OM, GP, PM, and QB designed and implemented the study, and gathered data and samples. SAL, CG, JR, and SC were mainly responsible for microbiological studies. SAL designed bacteriological laboratory techniques and supervised laboratory work at the University of Washington (Seattle, WA, USA). OM and EdL did the statistical analyses. OM and PM wrote the first draft of the report with revisions and input from AK, WH, WK, PS, and SAL. All authors contributed to revisions and approved the final version.

Delcaration of interests

We declare that we have no competing interests.

Acknowledgments

This study was supported by the Newcrest Mining Company. We thank the study participants and their parents or legal guardians, the field teams of the Lihir Medical Centre, and the staff at the Molecular Diagnostic Unit, Pathology Queensland, Brisbane, Australia who did the *H ducreyi* molecular testing.

References

1 Mitjà O, Asiedu K, Mabey D. Yaws. *Lancet* 2013; **381:** 763–73.
2 de Noray G, Capuano C, Abel M. Campaign to eradicate yaws on Santo island, Vanuatu 2001. *Med Trop* 2003; **63:** 159–62.
3 Fegan D, Glennon MJ, Thami Y, Pakoa G. Resurgence of yaws in Tanna, Vanuatu: time for a new approach? *Trop Doct* 2010; **40:** 68–69.
4 Marckmann P, Højbjerg T, von Eyben FE, Christensen I. Imported pedal chancroid: case report. *Genitourin Med* 1989; **65:** 126–27.
5 Ussher J, Wilson E, Campanella S, Taylor SL, Roberts SA. *Haemophilus ducreyi* causing chronic skin ulceration in children visiting Samoa. *Clin Infect Dis* 2007; **44:** 85–87.
6 Peel TN, Bhatti D, De Boer JC, Stratov I, Spelman DW. Chronic cutaneous ulcers secondary to *Haemophilus ducreyi* infection. *Med J Aust* 2010; **192:** 348–50.
7 McBride WJ, Hannah RC, Le Cornec GM, Bletchley C. Cutaneous chancroid in a visitor from Vanuatu. *Australas J Dermatol* 2008; **49:** 98–99.
8 WHO. Eradication of yaws—the Morges Strategy. *Wkly Epidemiol Rec* 2012; **87:** 189–94.
9 Mitja O, Hays R, Ipai A, et al. Outcome predictors in treatment of yaws. *Emerg Infect Dis* 2011; **17:** 1083–85.
10 WHO. Summary report of a consultation on the eradication of yaws. 5–7 March 2012. Geneva: World Health Organization, 2012.
11 Mitjà O, Hays R, Ipai A, et al. Single-dose azithromycin versus benzathine benzylpenicillin for treatment of yaws in children in Papua New Guinea: an open-label, non-inferiority, randomised trial. *Lancet* 2012; **379:** 342–47.
12 Autier P, Delcambe JF, Sangare D, et al. Etudes sérologiques et cliniques sur la tréponématose endémique en République du Mali. *Ann Soc Belge Méd Trop* 1989; **69:** 319–29.
13 Lukehart SA, Godornes C, Molini B, et al. Macrolide resistance in *Treponema pallidum* in the United States and Ireland. *N Engl J Med* 2004; **351:** 154–58.
14 Centurion-Lara A, Castro C, Shaffer JM, et al. Detection of *Treponema pallidum* by a sensitive reverse transcriptase polymerase chain reaction. *J Clin Microbiol* 1997; **35:** 1348–52.
15 Marra CM, Sahi SK, Tantalo LC, et al. Enhanced molecular typing of *Treponema pallidum*: geographical distribution of strain types and association with neurosyphilis. *J Infect Dis* 2010; **202**: 1380–88.
16 Centurion-Lara A, Giacani L, Godornes C, et al. Fine analysis of genetic diversity of the tpr gene family among treponemal species, subspecies and strains. *PLoS Negl Trop Dis* 2013; **7:** 2222.
17 Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 1996; **34:** 49–54.
18 Al-Tawfiq JA, Harezlak J, Katz BP, Spinola SM. Cumulative experience with *Haemophilus ducreyi* 35000 in the human model of experimental infection. *Sex Transm Dis* 2000; **27:** 111–14.
19 Plummer FA, D'Costa LJ, Nsanze H, et al. Clinical and microbiologic studies of genital ulcers in Kenyan women. *Sex Transm Dis* 1985; **12:** 193–97.
20 Bogaerts J, Kestens L, van Dyck E, Tello WM, Akingeneye J, Mukantabana V. Genital ulcers in a primary health clinic in Rwanda: impact of HIV infection on diagnosis and ulcer healing (1986–1992). *Int J STD AIDS* 1998; **9:** 706–10.
21 O'Farrell N, Hoosen AA, Coetzee KD, van den Ende J. Genital ulcer disease in men in Durban, South Africa. *Genitourin Med* 1991; **67:** 327–30.
22 Chua SH, Cheong WK. Genital ulcer disease in patients attending a public sexually transmitted disease clinic in Singapore: an epidemiologic study. *Ann Acad Med Singapore* 1995; **24:** 510–14.
23 Thirumoorthy T. The epidemiology of sexually transmitted diseases in Southeast Asia and the western Pacific. *Semin Dermatol* 1990; **9:** 102–04.
24 WHO Regional Office for the Western Pacific. Guidelines for the management of sexually transmitted infections in female sex workers. Manila, Philippines: World Health Organization Regional Office for the Western Pacific STI, HIV and AIDS Focus, 2002.
25 Trees DL, Morse SA. Chancroid and *Haemophilus ducreyi*: an update. *Clin Microbiol Rev* 1995; **8:** 357–75.
26 Spinola SM, Wild LM, Apicella MA, et al. Experimental human infection with *Haemophilus ducreyi*. *J Infect Dis* 1994; **169:** 1146–50.
27 Al-Tawfiq JA, Thornton AC, Katz BP, et al. Standardization of the experimental model of *Haemophilus ducreyi* infection in human subjects. *J Infect Dis* 1998; **178:** 1684–87.
28 Pillay A, Chen CY, Reynolds MG, et al. Laboratory-confirmed case of yaws in a 10-year-old boy from the Republic of the Congo. *J Clin Microbiol* 2011; **49:** 4013–15.
29 Workowski KA, Berman S; Centers for Disease Control and Prevention (CDC). Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010; **59:** 1–110.